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A Silencing Safeguard: Links between RNA Silencing and mRNA Processing in *Arabidopsis*

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RNA silencing is a genome defense mechanism used by many eukaryotic organisms to fight viruses and to control transposable elements. Work by Gregory et al. on *Arabidopsis thaliana* (in this issue of *Developmental Cell*) revealed a mechanism whereby the plant protects its endogenous messenger RNAs from undergoing RNA silencing and uncovered an unexpected role of the cap-binding protein ABH1 in miRNA biogenesis.

RNA silencing is a mechanism in which 21–24 nucleotide (nt) small RNAs guide the transcriptional or posttranscriptional repression of target loci through sequence complementarity (reviewed in Baulcombe, 2005). At the core of RNA silencing, a double-stranded RNA is converted into small interfering RNAs (siRNAs) by an RNase III enzyme Dicer, and the siRNAs guide argonuate-containing silencing complexes to DNA loci to result in heterochromatin formation or RNA transcripts to lead to RNA cleavage. RNA silencing is thought to have evolved to combat RNA viruses or selfish genetic elements. dsRNAs from a replicating virus trigger the production of siRNAs, which then guide an argonaute protein with endonuclease activity to degrade viral RNAs. Repetitive DNA and transposable elements are endogenous targets of RNA silencing. dsRNAs from these loci are produced as a result of convergent transcription or, in plants, *Caenorhabditis elegans*, and *Schizosaccharomyces pombe*, are converted from single-stranded RNAs by cellular RNA-dependent RNA polymerases (RDRs). The dsRNAs are processed into multiple siRNAs, which then recruit DNA or histone methyltransferases to homologous DNA loci to promote heterochromatin formation.

Many eukaryotic organisms have adapted RNA silencing to regulate the expression of protein coding genes at the posttranscriptional level. These organisms have evolved non-protein-coding genes that give rise to 21–24 nt small RNAs known as microRNAs (miRNAs). A miRNA guides a silencing complex to target mRNAs with sequence complementarity to lead to transcript cleavage or translational inhibition.

Plants have further adapted RNA silencing to regulate protein-coding genes through a class of siRNAs known as *trans*-acting siRNAs (Peragine et al., 2004; Vazquez et al., 2004). In *Arabidopsis*, a capped and polyadenylated transcript from a *TAS* locus is channeled into the RNA silencing pathway by a cleavage event triggered by an miRNA. The cleaved *TAS* transcripts are then copied into dsRNAs by RDR6, and the dsRNAs are converted to siRNAs by one of the DICER-LIKE (DCL) proteins. The *trans*-acting siRNAs regulate their target mRNAs in the same manner as do miRNAs.

Given the widespread sources of siRNAs in the *Arabidopsis* genome, a question arises as to how protein-coding loci are largely immune from being targeted to undergo siRNA formation. The answer appears to lie in RNA quality control, as demonstrated by the study by Gregory et al., (2008) (in this issue) and implicated by earlier studies that focused on transgenes (Gazzani et al., 2004; Herr et al., 2006).

Initially interested in ethylene signaling, Gregory et al. (2008) examined the genetic interaction between a mutation in the mRNA cap-binding protein ABH1 (Hugouvieux et al., 2001) and a mutation in *EIN5*, also known as *XRN4*, which encodes a 5′-to-3′ exonuclease that degrades uncapped mRNAs. While they found that *abh1* suppressed the ethylene-insensitive phenotype of *ein5*, they also noticed novel synthetic phenotypes from the two mutations that resemble those of small RNA biogenesis mutants. This led them to investigate the effects of the two mutations on the genomic landscape of endogenous small RNAs through small RNA profiling using the sequence-by-synthesis technology. They found that, in *ein5*, hundreds of protein-coding loci become sources of clusters of 21 nt siRNAs. The small RNAs appear to have been derived from both sense and antisense strands of the transcripts, suggesting that the transcripts from these loci have been converted to dsRNAs by a cellular RDR. Noncapped RNAs from these loci were found to accumulate in *ein5*, suggesting that EIN5/XRN4 degrades uncapped mRNAs to prevent them from being channeled into the RNA silencing pathway. A previous study found that EIN5/XRN4 degrades uncapped RNAs from a transgene and loss of *XRN4* renders the transgene more susceptible to RNA silencing (Gazzani et al., 2004). The conclusions from these studies are that (1) the 5′ cap of an mRNA protects the mRNA from undergoing siRNA production, perhaps by deterring a cellular RDR from using the mRNA as a template to make dsRNA, and (2) EIN5/XRN4 removes any uncapped mRNAs, which could serve as templates of a cellular RDR, to ensure that RNA silencing does not occur on most protein-coding genes.

Maintaining the integrity of one end of an mRNA is unlikely to be sufficient to prevent RNA silencing. A study by the Baulcombe group showed that mutations in an RNA splicing factor or several proteins acting in mRNA 3′ end formation result in enhanced RNA silencing of a transgene (Herr et al., 2006). This suggests that splicing and 3′ cleavage and polyadenylation are also crucial steps in RNA metabolism that prevent RNA silencing. Although endogenous transcripts were not examined in this study, it would not be surprising if certain endogenous

transcripts also undergo RNA silencing in these mutants.

Taken together, one model is that cellular mRNAs are monitored for their integrity (intact cap, polyA tail, correct splicing, polyadenylation, subcellular localization, etc.) such that ''abberant'' RNAs are degraded to prevent them from being converted into dsRNAs by cellular RDRs (Figure 1). *Arabidopsis* RDR6 protein is unable to distinguish between naked RNAs with or without a cap or polyA tail in vitro (Curaba and Chen, 2008). Therefore, proteins that are associated with an intact mRNA, such as cap-binding proteins, exon junction complexes, and polyA-binding proteins, probably prevent RDR6 from using mRNAs as substrates.

Gregory et al. (2008) also uncovered an unexpected role of the cap-binding protein ABH1 in miRNA biogenesis. miRNAs are processed from primary transcripts from *MIR* genes, known as pri-miRNAs. Pri-miRNAs are first cropped into transcripts forming stem-loop structures called pre-miRNAs, which are further processed into miRNAs. The two processing steps require DCL1, HYL1, a dsRNA-binding protein, and SERRATE (SE), a zinc finger protein (reviewed in Chen, 2008). A genome-wide transcript analysis showed that the levels of 19 pri-miRNAs increased in an *abh1* single mutant. Mature miRNAs, on the other hand, were reduced in abundance, but were not completely absent, in the *abh* single mutant. This suggests that ABH1 is important but not essential in the processing of pri-miRNAs. Consistent with a role in miRNA biogenesis, *abh1* plants have serrated leaves, a phenotype similar to hypomorphic *se* mutants (a null *se* allele is embryonic lethal) (Lobbes et al., 2006).

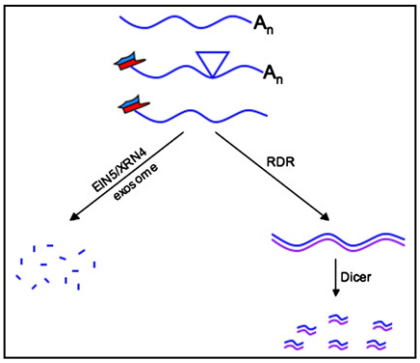

Figure 1. A Tug of War between RNA Surveillance and RNA Silencing
Defects in cellular mRNA metabolism such as capping, splicing, and 3′ end cleavage and polyadenylation can result in uncapped mRNAs, mRNAs with introns retained, or mRNAs without polyA tails. These RNAs can be degraded by mRNA surveillance mechanisms involving 5′-3′ exonucleases such as EIN5/XRN4, 3′-5′ exonucleases such as the exosome, and nonsense-mediated decay. If the defective mRNAs are not properly removed, they can be channeled into the RNA silencing pathway through the activities of cellular RDR and Dicer.

How does ABH1 promote pri-miRNA processing? One possibility is that ABH1 recruits capped pri-miRNAs to the DCL1/HYL1/SE processing complex by interacting with one of the three proteins. A second possibility is that ABH1 retains pri-miRNAs in the nucleus, or even channels pri-miRNAs to processing bodies in the nucleus, to facilitate the nuclear-based pri-miRNA processing events. With these two models, one remaining question is how ABH1 distinguishes pri-miRNAs from the large pool of capped transcripts in the nucleus. Another possibility is that ABH1 stabilizes pri-miRNAs to protect it from RNA decay. In this capacity, ABH1 does not need to distinguish pri-miRNAs from other capped RNAs.

In conclusion, proteins that play general roles in mRNA metabolism, such as cap-binding, splicing, polyadenylation, RNA export, and RNA stability, can influence the efficiency or specificity of small RNA pathways.

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